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Skeletal Microstructure of *Galaxea fascicularis* Exsert Septa: A High-Resolution SEM Study

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Abstract. The deposition of four crystal types at the growth surface of the septa of several color morphs of the coral *Galaxea fascicularis* was investigated over a 24-h period. Results suggest that nanocrystals, on denticles at the apices of exsert septa, may be the surface manifestation of centers of calcification. These crystals were also found on the septa of the axial corallite of *Acropora formosa*. The deposition of nanocrystals appears to be independent of diurnal rhythms. Internally and proximal to the septal apices, distinct clusters of polycrystalline fibers originate from centers of calcification and form fanlike fascicles. Upon these fascicles, acicular crystals grow and extend to form the visible fasciculi at the skeletal surface. Deposition of aragonitic fusiform crystals in both *G. fascicularis* and *A. formosa* occurs without diurnal rhythm. Nucleation of fusiform crystals appears to be independent of centers of calcification and may occur by secondary nucleation. Formation of semi-solid masses by fusiform crystals suggests that the crystals may play a structural role in septal extension. Lamellar crystals, which have not been reported as a component of scleractinian coral skeletons before, possess distinct layers of polyhedral plates, although these layers also do not appear to be associated with daily growth increments. The relationship of lamellar crystals to other components of the scleractinian coral skeleton and their involvement in skeletal growth is unknown.

Introduction

The microstructural components of the $CaCO_3$ skeleton from a wide range of scleractinian corals have been well documented (see Wainwright, 1963; Vahl, 1966; Sorauf, 1970, 1972, 1974, 1980; Wise, 1970, 1972; Chevalier, 1974; Jell, 1974; Constantz, 1986, 1989). However, descriptions of skeletal microstructure are inconsistent, reflecting differences in both interpretation and structural variation. The relationships between the various crystalline microstructures found on the surface and within the interior of the skeleton are not fully understood, although this is fundamental to an understanding of the origin of crystal formation, deposition and growth.

The basic structure of the coral polyp is a tubelike skeleton, or corallum, divided by longitudinal and horizontal partitions. Sitting in the top of this tube is the living polyp. The key elements of the corallum are the longitudinal divisions, the septa, which are joined laterally by the wall (theca) of the corallum. Those septa that extend above the top of the theca are referred to as exsert septa. Exsert septa are one of the primary sites of $CaCO_3$ deposition and skeletal extension in the scleractinian coral *Galaxea fascicularis* (Marshall and Wright, 1998). These elongated septa protrude upward from the wall of the corallite and encircle the oral disc (see Fig. 1). This arrangement allows for individual septa to be easily detached from the corallite, without significant damage, for subsequent investigation of the crystalline microstructure with scanning electron microscopy (SEM).

The internal structure of the coral skeleton has been primarily studied by light microscopy. It was established early (Ogilvie, 1896) that the internal structure of septa is composed of arrays of vertically elongated centers of calcification from which polycrystalline fibers extend radially to form fascicles. These fanlike systems are regarded as the basic building blocks of the skeleton. On the external surface, two major crystal types have been described. These are clusters of acicular crystals that form fasciculi and spindle-shaped crystals referred to as fusiform crystals. The latter have been suggested to be deposited with a diel periodicity and to be the site of nucleation of fasciculi (Gladfelter,

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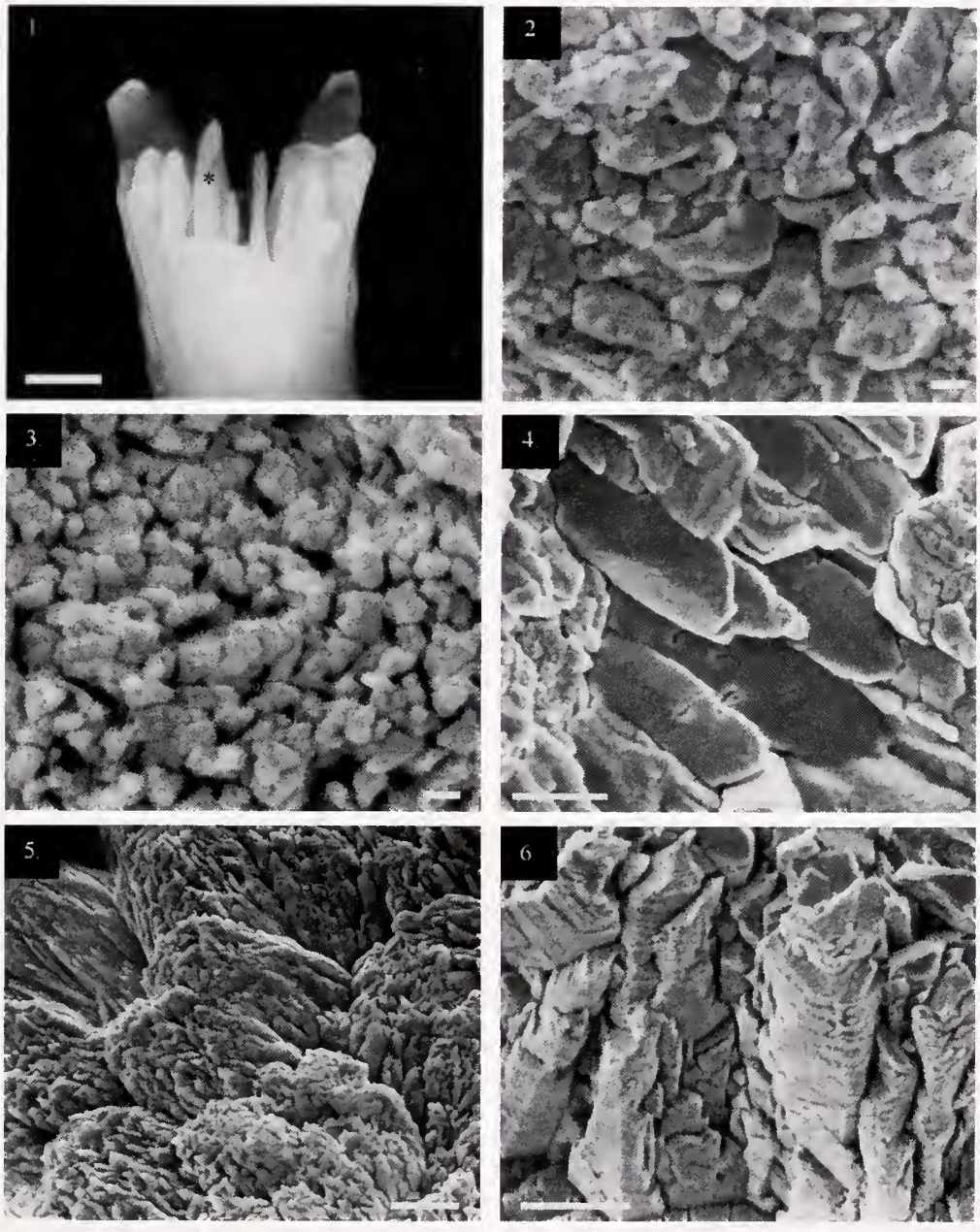

Figure 1. A corallite of *Galaxea fascicularis*, showing exsert septa (*) protruding from the wall of the corallite and encircling the oral disc. Scale bar = 2 mm.

Figure 2. Granular nanocrystals located upon the distal growth edge of a *Galaxea fascicularis* exsert septum. Scale bar = 100 nm.

Figure 3. Granular nanocrystals observed upon a septum of an axial corallite of *Acropora formosa*. Scale bar = 100 nm.

Figure 4. Highly ordered acicular crystals at the distal edge of a *Galaxea fascicularis* exsert septum. Scale bar = 500 nm.

Figure 5. Fasciculi, composed of distinct clusters of similarly oriented acicular crystals, constituting much of the distal surface of a *Galaxea fascicularis* exsert septum. Scale bar = 5 μm.

Figure 6. Lamellae crystals located proximal to the distal growth edge of a *Galaxea fascicularis* exsert septum. Scale bar = 1 μm.

1982, 1983). Fusiform crystals (Gladfelter, 1983) have been suggested to be calcite, in contrast to the bulk of the skeleton, which is formed from aragonite.

In this study we have investigated, over a 24-h period, the crystalline microstructure at the growth surface of exsert septa from the reef coral *G. fascicularis*. Structural charac-

teristics of four crystal types, including one crystal type not previously reported in scleractinian corals, are described at a new level, with magnifications greater than 50,000× achievable by low voltage, high-resolution field emission (FE) scanning electron microscopy. We find no evidence of rhythmic deposition of any crystal types. We also show by X-ray microanalysis that the composition of fusiform crystals does not appear to differ from the remainder of the aragonite skeleton.

Materials and Methods

Coral collection and maintenance

Green, yellow, and brown color morphs of colonies of the reef coral *Galaxea fascicularis* L. (different from the Japanese morphs described by Hidaka and Yamazato (1985)), and white-tipped branches of the reef coral *Acropora formosa* (Dana) were collected at low tide from the reef flat at Heron Reef in the Capricorn Bunker Group of the Great Barrier Reef, Australia. The corals were transported in buckets of seawater to the Heron Island Research Station, where they were maintained in sunlit, well-aerated flow-through aquaria in natural seawater at 24–25 °C. Following collection, the corals were allowed to recover for at least 2 days before being used for experimentation. On occasion, individual *G. fascicularis* polyps and *A. formosa* axial branches were sampled directly from the reef flat and immediately placed into the appropriate chemical treatment. *G. fascicularis* polyps of the green color morph were routinely used for all experiments.

Sample preparation for field emission scanning electron microscopy

Individual *G. fascicularis* polyps were sampled from colonies over a 24-h period (0600, 1200, 1800, and 2400 h; $n = 5$ for each time period). Individual polyps were easily separated from colonies with forceps, as the fragile coenosteum joining individual polyps could be removed without damaging the polyp itself. Axial tips of *A. formosa* branches were sampled at 1200 h ($n = 6$) and 2400 h ($n = 6$). All samples were placed in 12% NaOCl (commercial bleach) at 60 °C for 30 min, and the resultant corallites were rinsed well in running water and then in distilled water (dH_2O) several times. Any tissue remaining on the corallite was removed by gentle agitation and pipetting of dH_2O onto the sample, before being dried at 60 °C for 24 h (Clode and Marshall, 2003b).

G. fascicularis exsert septa (four septa from each polyp) were removed from the corallite with forceps, under a dissecting microscope, and mounted flat using carbon tape. Septa were coated with 5 nm platinum and previewed in a JEOL JSM 840A scanning electron microscope at 10 kV. High-resolution imaging was conducted on a JEOL 6340-F field emission (FE) scanning electron microscope at 2 kV. *A. formosa* branch tips ($n = 12$) were secured upright in hollow stubs with partially polymerized araldite, so that the axial polyp extended about 3 mm above the upper surface of the stub. Polymerization was then completed at 60 °C for a further 30 h. Conductive silver epoxy (ProSciTech) was used to improve the conductivity of the upright corallite, before it was coated with 10 nm platinum. Axial polyps were viewed in a JEOL JSM 6340-F FE scanning electron microscope at 1 kV and 2 kV.

All size measurements were obtained using the computer software package UTHSCSA Image Tool ver. 1.23 (University of Texas). All statistical analyses were performed using the computer software package JMP ver. 3.1.6 (SAS Institute, Inc).

X-ray microanalysis

For comparative elemental analyses of fusiform crystals and typical skeleton, *G. fascicularis* septa were mounted flat as described above, coated with 200 Å Al, and analyzed by X-ray microanalysis in a JEOL JSM 840A SEM fitted with a Link exL X-ray analyzer (Oxford Instruments). The analyzer was equipped with an LZ5 light element detector with a takeoff angle of 40°. Selected area analyses were conducted at 15 kV and a beam current of 2×10^{-10} A, from an area of 1 μm × 1 μm, for 100 s livetime. Element concentrations were calculated against microprobe reference standards (BioRad) using the PhiRhoZ model (Oxford Instruments) (Marshall, 1982; Marshall and Condron, 1987), and element ratios calculated. Because the X-rays from elements of interest could be generated from a depth of up to 2 μm at 15 kV, only large fusiform crystals were analyzed, reducing the likelihood that extraneous X-rays would be derived from skeleton below the crystal itself and affect the element ratios. The areas selected for analysis were horizontal relative to the X-ray detector.

Transverse slices

Small *G. fascicularis* polyps were rapidly frozen in liquid propane (−180 °C) and freeze-substituted in a mixture of 10% acrolein in diethyl ether, according to the protocol outlined by Marshall and Wright (1991). Transverse slices of freeze-substituted material, 400 μm thick, were prepared with a diamond saw (see Marshall and Wright, 1991), attached to glass slides with araldite, and then polished with aluminium oxide. The polished slices were rinsed in dH_2O and air-dried. Samples were viewed unmounted on a Zeiss Axioskop microscope with polarized light.

Results

Crystal types

Using low voltage, high-resolution FESEM, we identified four principal crystal forms on the growth surface of exsert septa sampled from polyps of *Galaxea fascicularis*. These

four crystal forms were nano, acicular, lamellar, and fusiform. All four types were common to septa collected at 0600, 1200, 1800, and 2400 h, and all appeared consistently similar in structure across the four sampling periods. Similarly, no notable differences were observed in the crystal structure of septa sampled from different color morphs, nor were there differences between corallites sampled directly from the reef flat and those allowed to recover in aquaria at Heron Island Research Station for 2 days.

Granular nanocrystals were commonly observed at the actively growing distal tip of *G. fascicularis* exsert septa predominantly on denticles. These crystals appeared as small, clustered groups of rounded crystals that exhibited little order in orientation or pattern of deposition (Fig. 2). Nanocrystals were also observed on septal surfaces of *A. formosa* axial corallites (Fig. 3). Nanocrystals were highly variable in size: the smallest resolvable crystals averaged 19 $\pm$ 0.8 nm (n = 12) in diameter, and the largest were about 400 nm in diameter.

Acicular crystals were the predominant crystal form on the surface of *G. fascicularis* exsert septa. These crystals were evident over much of the septal surface and extended perpendicular to the plane of the skeletal surface. Acicular crystals were typically large, solid crystals elongated along the c axis (Fig. 4), although smaller and more needlelike crystals were also observed. In contrast to nanocrystals, individual acicular crystals were elongated and exhibited a high degree of order and orientation. Groups of acicular crystals growing parallel to each other extended from an unseen origin, which was presumably the underlying fascicles. This arrangement resulted in the appearance of distinct clusters of similarly oriented acicular crystals termed fasciculi, visible at the skeletal surface (Fig. 5). No notable growth increments were evident within individual acicular crystals, suggesting a pattern of continuous growth.

Lamellar structures were typically observed in positions proximal to the extending distal edge of *G. fascicularis* septa. These crystals were similar to acicular crystals in that initially, at low magnification, they appeared to be large, elongate crystals extending perpendicular to the c axis. However, at high magnification it became evident that these were not single crystals, but layers of polyhedral plates resembling tabular crystals, which formed lamellar-like stacks (Fig. 6). These crystal stacks were distinctly different from acicular crystals: the apparent continuous nature of acicular crystal growth contrasted with the formation of distinct layers and the obvious discontinuous pattern of crystal deposition in lamellar stacks. Individual crystal layers within these stacks were less than 100 nm in thickness, whereas the crystal stacks themselves were highly variable in both height and diameter.

Fusiform crystals were observed principally along the lateral edges of *G. fascicularis* exsert septa (Fig. 7) and upon *A. formosa* primary septa extending into the calyx of axial corallites (Fig. 8). These crystals were regularly observed on all coral samples, regardless of time of sampling. Fusiform crystals appeared as large, tapered structures that were usually clustered together to form a semisolid, crystalline mass along the lateral edges of the septa (Fig. 7). In *G. fascicularis*, these crystals averaged 4.6 $\pm$ 0.2 μm in length and 2.3 $\pm$ 0.1 μm in width (n = 28). Fusiform crystals observed on *A. formosa* axial corallites were significantly shorter (3.7 $\pm$ 0.2 μm; n = 28; $P < 0.01$; Student's t test) and narrower (1.6 $\pm$ 0.1 μm; n = 28; $P < 0.0001$; Student's t test) than those on *G. fascicularis* septa.

Using high-resolution FESEM, we determined that fusiform crystals were not monocrystalline, but were instead composed of small, polycrystalline aggregates 21 $\pm$ 0.8 nm (n = 20) in diameter (Fig. 9). These spherical crystals upon the surface of fusiform crystals were not dissimilar to the nanocrystals at the growth edge. Needlelike crystals were never observed upon, or seen to be extending from, the surface of fusiform crystals in either *G. fascicularis* or *A. formosa*.

The ratios of the three major skeletal elements, Ca, Sr, and Mg, present in individual fusiform crystals and typical skeleton of *G. fascicularis* exsert septa, determined by X-ray microanalysis, revealed that fusiform crystals were of a very similar elemental composition to the main skeletal component. Differences ($P > 0.05$; Student's t test) observed in the Ca:Mg ratio, the Sr:Mg ratio, and the Ca:Sr ratio between fusiform crystals and skeleton were highly insignificant (Table 1).

No differences were observed, with respect to microstructure or chemical composition, between color morphs of *G. fascicularis* or between corals processed immediately on collection from the reef and corals processed after being kept in aquaria for 2 days after collection.

Freeze-substituted transverse slices

The major structural components of septa were clearly visible in transverse sections of whole freeze-substituted *G. fascicularis* polyps visualized with polarized light (Fig. 10). Centers of calcification, which appeared as distinctly darker (denser) regions, were evident along the midline of each septum. The closeness of the centers to each other and the thickness of the section made it difficult in a single focal plane to resolve the centers as separate structures; this was possible, however, when the plane of focus was changed. The centers possessed a granular substructure, but again, because of the thickness of the section, this was difficult to illustrate photographically. These centers of calcification extended along the central region of each septum, ceasing just short of the lateral edges. From these centers of calcification, highly ordered fascicles with distinct orientations radiated outwards to form fanlike systems (Fig. 10). Bundles of acicular crystals that form fasciculi at the septal surface cannot be visualized.

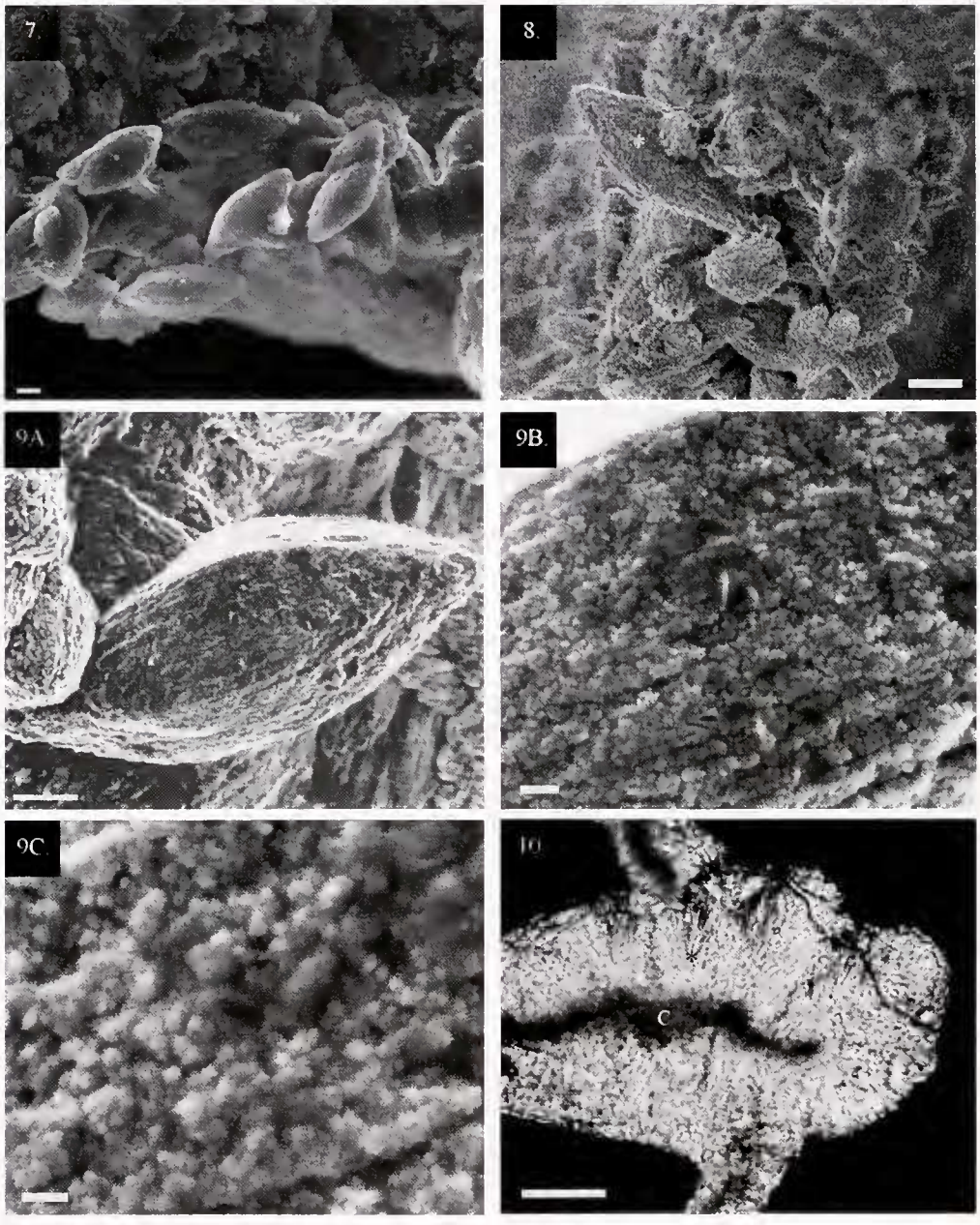

Figure 7. Clusters of fusiform crystals forming a semisolid crystalline mass along the lateral edge of a *Galaxea fascicularis* exsert septum. Scale bar = 1 μm.

Figure 8. Fusiform crystals (*) upon a primary septum extending into the calyx of an *Acropora formosa* axial corallite. Scale bar = 1 μm.

Figure 9. An isolated fusiform crystal from a *Galaxea fascicularis* exsert septum, shown in increasing magnifications. At high magnification, the surface of the tapered polycrystallite is seen to be covered in small, spherulitic crystals. Scale bars: A = 1 μm; B = 200 nm; C = 100 nm.

Figure 10. Transverse section through a freeze-substituted *Galaxea fascicularis* septum, viewed with polarized light, showing granular centers of calcification (C) and centrically arranged fascicles (*) radiating from these centers of calcification to form fanlike trabeculae. An array of trabeculae forms the entire septum. Scale bar = 100 μm.

Discussion

The most significant finding of this investigation is the presence of nanocrystals at the major growth points—the denticles—on the skeletal septa of *Galaxea fascicularis*. These nanocrystals may represent nucleation sites for the deposition of acicular crystals that ultimately form fanlike systems, or fascicles, of polycrystalline fibers, which are the major building blocks of the skeleton. Also found on septa were clusters of acicular crystals forming fasciculi, fusiform

Table 1

Comparison of the ratios of the primary skeletal elements present in individual fusiform crystals and typical skeleton from Galaxea fascicularis *exsert septa, as determined by X-ray microanalysis*

	n	Ca:Mg	Sr:Mg	Ca:Sr
Skeleton	6	143:1	2:1	76:1
Fusiform crystals	7	134:1	1.9:1	76:1
P Value*		0.77	0.82	0.99

* Student's *t* test; *n* = number of analyses.

crystals, and a novel lamellar type of crystal. None of these crystals appeared to be deposited in a diurnal rhythm. The structure of the exsert septum and the structure and location of the various crystal types is summarized in Figure 11. No differences were observed between different color morphs or between corals processed immediately on collection and corals maintained in aquaria for 2 days before processing.

Crystal types

Granular nanocrystals as small as 19 nm in diameter were observed by FESEM on the apical denticles of *G. fascicularis* septa. These nanocrystals have not been previously described. They may be the basic elements of centers of calcification, since denticles are the terminations of trabecular axes, which are extended centers of calcification in the septa (Ogilvie, 1896). Nanocrystals were also observed on the septa of the axial corallites of *A. formosa*. The nanocrystals appear to have some similarity to the crystals forming the nuclear packets described by Constantz (1989) and to the granulated crystallites upon the surface of "spherular crystals" noted by Isa (1986). Constantz suggested that

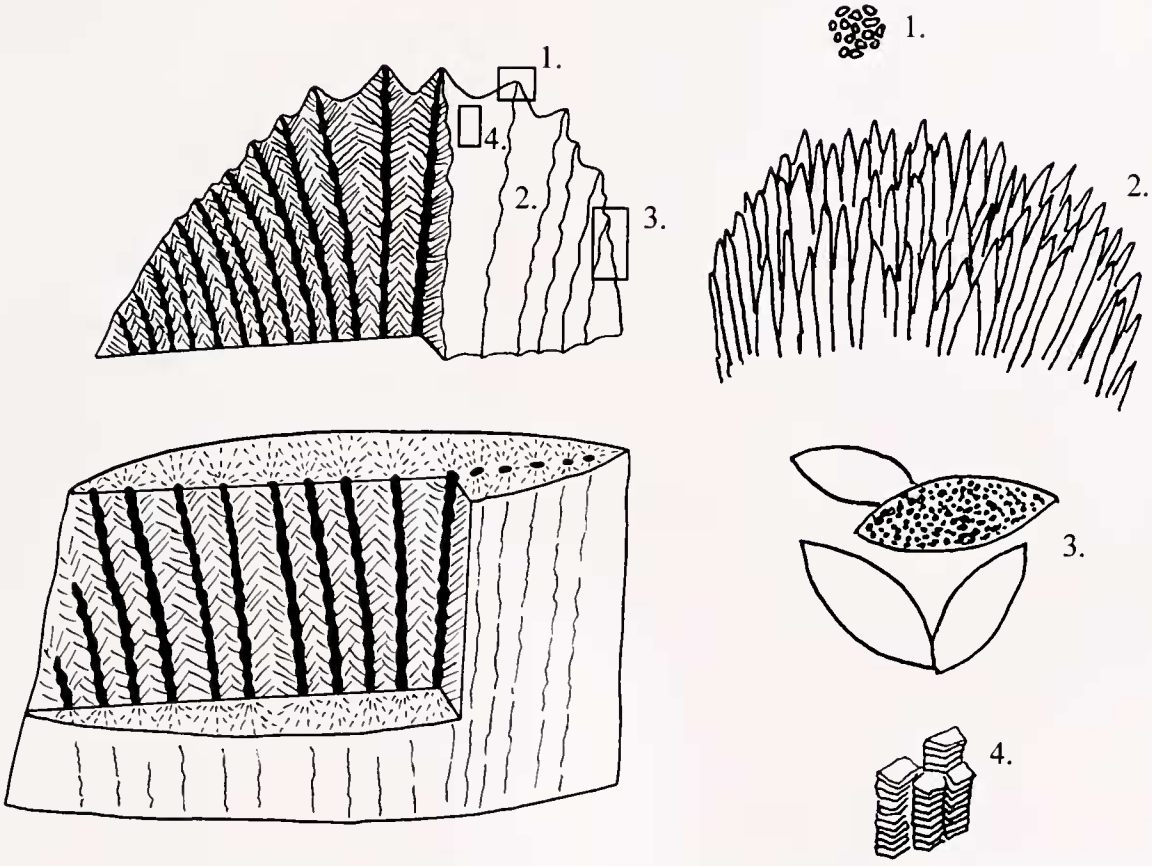

Figure 11. Diagrammatic representation of an exsert septum of *Galaxea fasicularis* showing the internal structure and distribution of crystal types. The internal structure is based partially on Ogilvie (1896). The diagram represents the upper part of the exsert septum with a transverse slice removed. The exsert septum is orientated so that the interior of the corallum would be to the left. Centers of calcification are depicted in black and terminate at denticles. Fibers form systems of trabecullae around the centers of calcification. New centers of calcification appear as the exsert septum extends. The different crystal types are drawn approximately to scale relative to each other, and their typical locations are indicated. Granular nanocrystals (1) are located at the growth edge (see Fig. 2). Acicular crystals (2) are widely distributed on the septal surface (see Figs. 4 and 5). Fusiform crystals (3) are found at the lateral edge (see Fig. 7), and lamellar crystals (4) are located close to the distal growth edge of the exsert septum (see Fig. 6).

nuclear packets were centers of calcification, describing them as small clusters of tiny crystals less than 100 nm in size that existed in high frequency near rapidly growing regions.

The mechanisms involved in the formation and deposition of the nanocrystals, so that they may act as nucleating centers for future crystal growth, remains unknown. There is evidence, however, that small nascent crystals of $CaCO_3$ may develop upon a fibrillar organic matrix, which is evident within small pockets formed between calicoblastic ectodermal cells and the pre-existing skeleton (Clode and Marshall, 2003a).

Clusters of acicular crystals form the distinctive fasciculi, which are visible on the surface of septa in *G. fascicularis*. The surface of coral skeleton is frequently characterized by groups of nearly parallel acicular crystals termed fasciculi (Wise, 1972). Depending upon the orientation of the crystals within the fasciculi, the skeletal surface may appear to be granular or relatively smooth. The acicular crystals presumably nucleate and extend from the apical edges of fascicles. Fascicles are fanlike systems of polycrystalline fibers radiating from centers of calcification (Ogilvie, 1896). The relationship between the smaller fasciculi and the larger underlying fascicles is not clear, since individual fasciculi cannot be readily recognized below the skeletal surface (Jell, 1974). Presumably, fasciculi give rise to the underlying fascicles; however, fascicles are also present in corals that do not have fasciculate skeletal surfaces (Wise, 1972).

Fusiform crystals were predominantly observed along the lateral edges of the septa. The term "fusiform" was first coined by Gladfelter (1982, 1983) to describe large, tapered crystals found on the growing surface of *Acropora cervicornis* axial corallites. Hidaka (1988) also employed the term to describe similar crystals observed on *G. fascicularis* exsert septa. Our observations on the size and shape of fusiform crystals from the septa of both *A. formosa* and *G. fascicularis* are consistent with these studies. The significant size differences evident between the fusiform crystals of *G. fascicularis* and *A. formosa* are likely to reflect differences in polyp size, with *G. fascicularis* polyps considerably larger than those of *A. formosa*. Earlier studies have also reported similar crystals, but these were described as "equant" crystals (see Constantz, 1989), while Isa (1986) preferred to use the term "spindle-shaped crystals." Le-Tissier (1988) also reported fusiform crystals upon the surface of *Pocillopora damicornis* corallites, but these lacked the characteristic tapered ends and may be a different crystal type.

Fusiform crystals on *G. fascicularis* septa were typically observed at the lateral edges where centers of calcification do not persist (Cuif and Dauphin, 1998). This is consistent with the suggestion of Constantz (1989) that centers of calcification were not required for nucleation and growth of fusiform crystals. Fusiform crystal formation may result from secondary nucleation, which can occur due to the presence of already existent crystal structures (Simkiss, 1986).

Upon the surface of fusiform crystals it was possible to resolve small spherical nanocrystals that were, on average, 21 nm in diameter. Isa (1986) reported that the surface structure of spindle-shaped (fusiform) crystals was composed of clusters of small, rounded crystals less than 50 nm in size, indicating that fusiform crystals were polycrystalline in nature. Isa (1986) also found that the fusiform crystals were hollow; however, the preparations had been treated with osmium tetroxide, which will react with $CaCO_3$ to cause dissolution and recrystallization.

We observed no evidence of acicular crystal growth upon individual fusiform crystals, contrary to the proposal of Gladfelter (1982, 1983) that clusters of needlelike crystals extended from fusiform crystals to ultimately form fasciculi. Instead, large clusters of fusiform crystals were typically cemented together to form a semisolid crystalline mass, a feature also noted by Hidaka (1991b), which bore little resemblance to the distinctive fasciculi. In addition, fasciculi, which were common to the entire septal surface, may be spatially isolated from fusiform crystals, which were typically confined to the distal (Hidaka, 1991a) or lateral edges. Hidaka (1991b) also recognised this paradox and suggested that fasciculi may form in several different ways.

Variability in the reported distribution of fusiform crystals on septa (Gladfelter, 1982, 1983; Hidaka, 1991a,b; Hidaka and Shirasaka, 1992) has made interpretation and understanding of crystal deposition and skeletal extension in corals difficult. Reasons for these reported differences are unknown, but preparatory techniques and environmental conditions may have significant effects upon skeletal microstructure (Carlson, 1999; Clode and Marshall, 2003b).

To our knowledge, lamellar crystals have not been reported as a component of any recent scleractinian coral skeleton. There is some suggestion that lamellar structures are existent in hydrozoans and tabulate and rugose anthozoans (see Wendt, 1990), although these appear to refer more to the orientation of fibrillar-type crystals than to true crystalline stacks of polyhedral plates. Lamellar stacks composed of polyhedral plates are very common in molluscs (Watabe and Dunkelberger, 1979), particularly in *Nautilus* shell nacre (Gregoiré, 1987). While molluscan lamellar structures may be either aragonite or calcite, lamellar crystals on mature *G. fascicularis* skeletons are likely to be aragonitic, as calcite persists only in the developing skeletal elements of coral larvae. The function of these lamellar structures in scleractinian corals is unknown, as is their relationship to other crystal types and their involvement in the overall extension and growth of skeletal elements.

Compositional analysis of fusiform crystals

X-ray microanalysis of individual fusiform crystals suggests that fusiform crystals are identical to skeleton in

element composition; therefore, they are aragonitic and not calcitic in nature. Constantz (1989) also suggested that fusiform crystals were likely to be composed of aragonite, although he provided no supporting evidence. Gladfelter (1982), using X-ray microanalysis of large areas of the skeleton of *Acropora cervicornis*, found that Mg concentrations were higher in areas where fusiform crystals were common than in other regions of the skeleton. Since calcite has a higher proportion of Mg than aragonite, it was suggested that fusiform crystals were composed of calcite. However, under these circumstances, it would be impossible to determine exactly what was analyzed, with the presence of fusiform crystals in each region of analysis not confirmed.

Diurnal rhythms

All four crystal types found on the exsert septa of *G. fascicularis* were present and remained similar in structure and disposition, regardless of time of sampling over a 24-h period. Apparent diurnal rhythms of crystal deposition have been reported in *Plesiastrea versipora* (Howe and Marshall, 2002), *Acropora cervicornis* (Gladfelter, 1983), *Pocillopora damicornis* (LeTissier, 1988), and *Manicina areolata* (Barnes, 1972). Hidaka (1988) initially reported a diurnal pattern of fusiform crystal deposition in the exsert septa of *G. fascicularis* corallites, but he later retracted this interpretation in favor of crystal deposition being without rhythm (Hidaka, 1991a). Similarly, we report that *A. formosa* axial corallites, whether sampled at 1200 or 2400 h, possessed fusiform crystals along the primary septa extending into the calyx. This finding is not in accordance with that of Gladfelter (1983), who only observed fusiform crystals upon axial corallites of *A. cervicornis* branches sampled in darkness.

Gladfelter (1982, 1983) hypothesized that fusiform crystals form a loose scaffolding on the surface of exsert septa at night and that acicular crystals nucleate on the fusiform crystals during the day, ultimately giving rise to fasciculi. This diel cycle of deposition of fusiform crystals was proposed to account for skeletal extension in zooxanthellate corals at night. However, diel deposition of fusiform crystals is apparently not a universal phenomenon (*e.g.*, Hidaka, 1991a), and such crystals are not present in all corals (*e.g.*, Howe and Marshall, 2002).

The universal presence of acicular crystals as a predominant component of scleractinian coral skeletons during both day and night, in combination with their lack of discernible substructure, suggests that the growth of these crystals is continuous. Whether the rate of crystal extension and growth varies throughout the day is unknown, but diurnal variations in skeletal extension have been reported (Barnes and Crossland, 1980). In contrast, the presence of distinct layers within lamellar stacks suggests an intermittent, highly regulated process of crystal deposition. Each layer is likely to represent growth increments; however, as no intermediate stages of deposition were observed over the 24-h sampling period, these layers do not appear to be associated with a daily pattern of crystal deposition and growth.

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